

Sperm competition in *Pholcus phalangioides* (Fuesslin, 1775) (Araneae, Pholcidae)—shorter second copulations gain a higher paternity reward than first copulations

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Summary

Sperm competition has been established as an important evolutionary force shaping mating systems in many animal groups. A measurement of the typical outcome of sperm competition between two males is useful because it can explain a suite of peri-reproductive behaviours. The measure is expressed as the proportion of a brood sired by the second mating male (P_2). The P_2 was measured in the haplogyne spider *Pholcus phalangioides* because it has been hypothesized that the sperm storage organ should favour a high P_2 in comparison with the few entelegyne spiders already studied. This spider is particularly suited to studies of this kind because its reproductive biology is well known and because sperm competition is important in the wild in this species. As predicted by the theory, a high P_2 (0.62, range 0.0–1.0, SD = 0.35, which is significantly greater than 0.5 ($P = 0.018$) assayed from 833 individual progeny, in 28 broods, laid by 10 females) was found in this species. However, the large range and trend of P_2 across broods were incompatible with other predictions of the theory. The high P_2 was recorded despite the significantly shorter mating duration of second mating males compared with males mating first. Reasons for this apparent paradox of paternity reward are explored.

Introduction

Ever since Geoff Parker's (1970) seminal paper, it has been recognized that sperm competition is of prime importance in the mating systems of animals. Sperm competition occurs when ejaculates of more than one male are present in the reproductive tract of a female within a reproductive period. The consequences for male peri-reproductive behaviour are far reaching: on the one hand, they may try to avoid sperm competition by, for instance, mate guarding or, on the other, try to usurp paternity when copulating with a mated female by removing their rivals' sperm.

Austad's (1984) review has provided the context for studies of sperm competition in spiders for over a decade. He proposed that the female's sperm storage organ (SSO) may, depending on its structure, bias paternity allocation over her

brood(s) to the first or the last male. If the SSO is cul-de-sac in construction (approximately equivalent to Haplogynae, where only one duct serves for entry and exit of sperm) then the second male is favoured. Conversely, conduit SSOs (approximately equivalent to the Entelegynae, where two ducts serve the SSO) are meant to favour the first mating male. These predictions are based upon the assumption that the sperm stratify within the SSO. Clearly, stratification has different consequences in the different types of SSO. In the cul-de-sac the last male's sperm is likely to be the first out because it overlays the first male's, resulting in a high P_2 (the proportion of the brood sired by the second mating male). In the conduit, where there is a separate exit, the first male's sperm is more likely to enter the fertilization set (low P_2).

There is a body of empirical evidence in favour of this position (reviewed by Elgar, in

press). However, two studies (Masumoto, 1993; Andrade, 1996) on conduit species cast some doubt on the hypothesis, questioning its role as an organizing paradigm.

To probe this idea further, the haplogyne spider *Pholcus phalangioides* (Fuesslin, 1775) was selected with the aim of assessing the importance of sperm competition in wild populations and to attempt to refute the Austad (1984) hypothesis. There are a number of reasons why this species was suitable for this study:

1. Adults in breeding condition are available almost all year round.

2. They are anthropophilic in nature, so laboratory conditions are similar to their wild habitat (cellars) and therefore experiments in captivity will have a bearing on how they behave in the wild. This life-style also makes them easier to feed and rear than most spiders.

3. The reproductive physiology and behaviour of this species are well known (Uhl, 1994; Yoward 1996; Bartos, 1998). *P. phalangioides* has no specialized spermathecae, but the sperm storage location was unambiguously classified as cul-de-sac by Austad (1984) because there is only one entrance and exit for sperm.

4. Sperm competition is likely to be of importance in the life history of *P. phalangioides* because females are receptive to matings after the first (Yoward, 1996 and references therein) and the males do not guard them (G. Uhl & N. Reagan, pers. comm.). Additionally, sperm can be stored for a long time and it remains viable throughout storage (Uhl, 1993a) which means there is a high chance of ejaculate overlap.

Methods and results

Because wild-caught females' broods were to be analysed for the incidence of multiple paternity, a molecular paternity assignment technique was necessary. The most cost-effective method was judged to be allozyme electrophoresis. The conditions which resolved suitable scorable bands were found to be broadly similar to those used by Oxford (1986), except for staining for esterase, as described in Yoward (1996). Stained bands were achieved by dissolving the substrate alpha-naphthyl acetate in a drop of acetone, and then staining with Fast Garnet GBG, prepared as a saturated solution in 10 ml of reservoir buffer

(Den Boer, 1978). These two solutions were made up to 150 ml of reservoir buffer and the gel incubated in it for 30–60 minutes at room temperature. When the bands were resolved, the solution was washed off and the gel fixed with 50% ethanol.

There were three stages in the experimental procedure:

STAGE 1: Eight virgin females were each mated to a single virgin male to investigate the heritability of the bands in the subsequent progeny. Three loci were detected. No simple genetic interpretation could be found for the system migrating the furthest from the cathode. However, the genetics of the other two were both effectively consistent with the interpretation that they were simple diallelic systems (Yoward, 1996). None of the broods, consisting in total of 247 young (averaging over 30 young per brood), deviated from expected Mendelian ratios from the band genotypes of the parents.

STAGE 2: These markers were then used to investigate the incidence of multiple paternity in broods of 16 females collected from several sites in Europe. The females were isolated on delivery and allowed to lay up to 3 cocoons each (29 cocoons and 774 young in all). This meant there were two ways to discover if a female had mated with more than one male: (1) a deviation from a Mendelian ratio within a brood; and (2) a different type of Mendelian ratio between broods laid by a single female. In 6 out of the 16 females, evidence for multiple paternity existed. For several reasons this is probably an underestimate of the degree of polyandry in the wild: (1) only two loci were scored, which means that a small range of possible genotypes for the different males at a site exists and the males have to be a different genotype to be distinguished—at some sites this is particularly unlikely because preliminary sampling provided evidence that the adults at those sites were fixed for a particular allele; and (2) some broods were small ($n < 15$), which means that a deviation from a Mendelian ratio is statistically unlikely.

STAGE 3: Sixty virgin females were each observed to mate successfully with two sequentially presented virgin or mated males. Ten of the females were allowed to lay up to 4 cocoons, the young of which ($n = 833$ from 28 cocoons) were harvested separately by brood. These matings were used to calculate a P_2 value for

P. phalangioides. The P_2 was found to be high overall (mean = 0.62; median = 0.72) and significantly different from 0.5 (the figure if random sperm mixing of equivalently sized ejaculates was the case, Nonparametric One-tailed Sign Test, $P = 0.018$), but with an extreme degree of variation (range = 0.0–1.0, SD = 0.35).

Although in the majority of cases (20/28—Table 1) the bulk of the paternity accrued to second mating males, the matings with virgin females were significantly longer (mean = 4450 seconds, range = 1152–7740) than those with mated females (mean = 1517 seconds, range = 85–6348, Wilcoxon Rank Sum test, $W = 1302$, $P < 0.0001$). This means first mating males are apparently mating for a prolonged duration without an extra fitness reward for their copulatory courtship (Eberhard, 1996). The mating experience of the males did not affect mating duration ($W = 759$, $P > 0.05$).

If sperm stratification within the SSO is the mechanism whereby paternity is determined, then one would expect a trend towards a lowering of the P_2 across broods laid by the same female as sperm supplies of the second mating male are used up. Inspection of Table 1 shows that this is not the case.

Discussion

Austad's prediction of a high P_2 has been borne out in the results of this study on *P. phalangioides*. This result contrasts with the mainly low P_2 s found in the entelegyne spiders studied so far. Previous studies of haplogynes found either little influence of the SSO on the P_2 (Eberhard *et al.*, 1993) or a strong second male advantage (Kaster & Jakob, 1997). However, the latter study, as well as this one, found a large variation around the mean high P_2 value. This facet was not predicted by the Austad hypothesis, nor was the lack of a trend in first male fertilization success across broods, which should increase as the second male's sperm are used up, if the sperm were stratified in the SSO. This suggests a mechanism of paternity determination in this species, other than that suggested by Austad (1984), when a female mates with more than one male. This may be because of the lack of a specialized SSO in *P. phalangioides*, which is thus not a typical cul-de-sac or haplogyne species.

Brood:	i	ii	iii	iv	Trend
1	0.56	0.08	0.00	–	Down
2	–	0.00	–	–	
3	–	1.00	1.00	–	Level
4	0.88	0.75	0.76	0.89	Level
5	–	0.16	0.58	–	Up
6	1.00	0.57	0.00	0.00	Down
7	0.66	1.00	1.00	0.88	Level
8	0.44	0.80	0.86	1.00	Up
9	0.39	0.84	–	–	Up
10	0.68	0.60	–	–	Level
Av:	0.66	0.58	0.60	0.69	–

Table 1: P_2 values calculated from diagnostic loci. Heterogeneity χ^2 tests were calculated on the original allozyme phenotype data to discover if there was a trend in the broods' P_2 values. If homogeneous they are reported as level, otherwise they are reported as a trend: up or down.

The most puzzling aspect of the results is the high reward to second copulations, despite their very much shorter duration. This result is in contrast with Uhl's (1993b) speculations as to whether sperm transfer from second matings occurred at all. A further question is why first matings are so long in these tripartite matings if they secure less paternity than second mating males in a mating system that appears to be promiscuous. There are three possible reasons for this: (1) Laboratory artefact; the second matings were set up temporally close to the first matings and this may be uncommon in the wild. If this is so, then changes in the female reproductive tract after the first mating may make second copulations difficult and give second matings little reward when there is a delay. The long first matings would then be explained on the grounds of contact mate guarding for the period within which most of these physiological changes take place or are induced by the male. The incidence of multiple paternity in wild collected females may only occur when a female remates to refill depleted sperm stores after already laying a cocoon. This suggests that the physiological mechanisms are reversible. Only one second mating experiment was carried out with some delay after the first mating and this had a lower than average P_2 for all broods. Further experiments are required to determine if P_2 scores are depressed by a longer intermating period. (2) The spermathecae may not have an

influence on priority. Second males may gain priority not because of positional factors but by force of numbers—the outcome of sperm competition thus determined by the “raffle principle” (*sensu* Parker, 1984). Second males may be able to secure more sperm in the female’s SSO either by passing on more sperm at copulation or by passing a similar amount but removing the sperm of the first male before transfer. This would not explain why second matings are shorter than the first, but the complex pedipalp structure in *P. phalangioides* compared to most haplogynes (Uhl *et al.*, 1995) is interesting in these respects and may have a sperm removal function. (3) First mating males may “expect” supernumerary matings by the female, and as Zeh & Zeh (1994) have shown in another species, last-male sperm priority may break down when more than two males mate with a female, in which case the lengthy mating by the first male may pay off.

As sperm competition studies on spiders accumulate it seems increasingly clear that Austad’s elegant model is becoming untenable. Despite a high P_2 found in this study, trends across broods and high variation do not comply with the model’s predictions. However, the female reproductive tract, because it is the environment in which sperm competition is fought out, may have some influence on its outcome in a polyandrous system, though it may not have an overriding one and male influences may interact with it.

Acknowledgements

I thank Geoff Oxford for supervision of the D.Phil., of which this work formed a part, and for securing an SERC grant which made the work possible. Gabriele Uhl and John Dalingwater substantially improved the manuscript with helpful comments, for which many thanks. Matthew Clegg provided grammatical guidance, though I accept responsibility for any errors.

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